Multi-locus sequence typing for phylogeny and fine typing of *Mycoplasma agalactiae* strains

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Introduction

Mycoplasma agalactiae is considered the strict agent of contagious agalactia, a syndrome affecting small ruminants that is responsible for great economic losses (OIE list). Control measures based on either stamping out and quarantine (France) or vaccination campaigns (Spain) have not prevented the appearance of residual outbreaks and a fine typing tool is required to asses whether these outbreaks are due to reappearance or to reintroduction of the causative agent. We have developed a Multi-Locus Sequence Typing (MLST) tool similar to that already used for the *M. mycoides* cluster (1), which has the advantage of allowing phylogenetic studies. *M. agalactiae* was once grouped with *M. bovis* into a single species. They share phenotypic and genotypic traits, which complicates diagnosis. However, 16S rDNA sequences are not suitable for phylogenetic analysis of these closely related species due to low interspecies and high intraspecies variability, coupled to high inter-operon polymorphism within strains (2).

Group of strains	Sequence length (bp)	Variable sites (nt)	Variability (%)	N° of sequence types
23 strains of the *M. mycoides* cluster	2376	206	8.67	19
7 *M. agalactiae* & 7 *M. bovis* strains	2619	529	20.20	11; 7 *M. agalactiae* & 4 *M. bovis*
19 *M. agalactiae* strains (validation)	2619	325	12.41	14

Table 1: Variability amongst different groups of strains analyzed by MLST

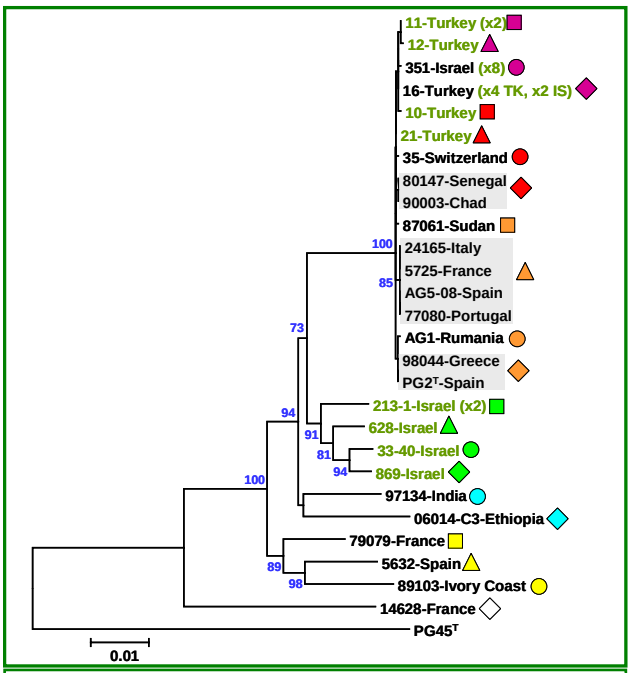


Fig. 3: Phylogenetic tree (Neighbor-Joining, MEGA) obtained by analysis of 19 validation strains (in black) and additional strains from Turkey and Israel (in green). Grayed boxes group identical sequences. Sequence types are represented by colored symbols.

Results and Discussion

Phylogenetic analysis:

M. agalactiae and *M. bovis* could be clearly resolved in a robust phylogenetic tree (Fig. 2), and intra-species variability was much higher than that observed within the *M. mycoides* cluster (Table 1). Still, atypical strains could be well positioned. All *M. agalactiae* strains could be discriminated.

M. agalactiae *typing*:

MLST of 19 *M. agalactiae* strains from diverse origins confirmed a great intraspecies variability (Table 1, Fig. 3). In contrast, a predominant group of strains showed very limited variability (0.46%). Fourteen sequence types were obtained that were not correlated to geographic origins (Fig. 4). However, no data was available to rule out epidemiological links between these strains.

The analysis of strains from Israel and Turkey revealed differences in *M. agalactiae* diversity in the two countries (Fig. 3): All 5 sequence types identified in Turkey were located within the main group of very similar strains. On the contrary, although 2 out of 6 sequence types from Israel also belonged to this main group, the other 4 formed a separate group showing greater variability (2.44%). Each country showed a predominant type that seems to have persisted over years.

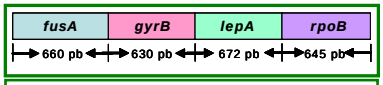
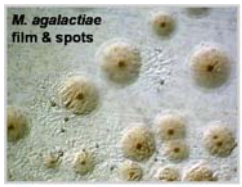


Fig. 1: Concatenated sequences for tree construction



Material and Methods

Four housekeeping gene sequences were concatenated for tree construction, (Fig. 1). Seven strains from diverse geographic origins belonging to each of the two species were initially used for phylogenetic analysis. Nineteen *M. agalactiae* strains representing the geographic distribution of this agent were then analysed to evaluate the discriminatory power of the MLST tool. Additional strains were used to analyze the diversity of *M. agalactiae* in Turkey (N=9) and Israel (N=15).

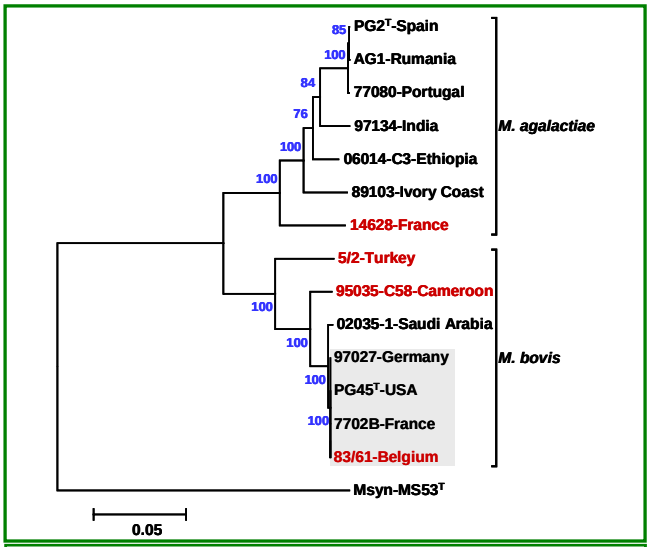


Fig. 2: Phylogenetic tree (Neighbor-Joining, MEGA) obtained by analysis of 7 strains from each species. Grayed boxes group identical sequences. Atypical strains, for which classical and / or PCR diagnosis was conflicting, are shown in red.

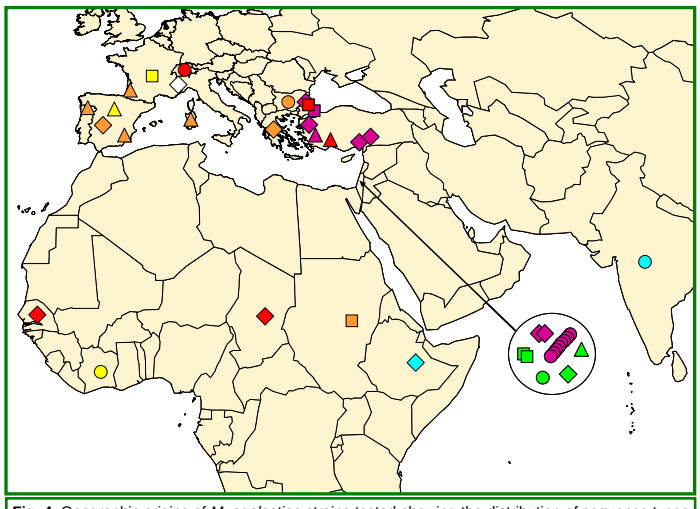


Fig. 4: Geographic origins of *M. agalactiae* strains tested showing the distribution of sequence types as shown in Fig. 3. Israeli strains are presented in a circle.

Conclusion

MLST reveals a high diversity within *M. agalactiae* that is related to the evolution of the strains. The presence of a group of closely related strains showing a wide geographic distribution may be explained by circulation of animals and clonal expansion of strains. Representative samples of strains accompanied by all related epidemiological data will be essential to conduct molecular epidemiology studies.

REFERENCES:
(1) **Konigsson *et al*. (2002)** *Vet Microbiol* **85**, 209-220
(2) **Manso-Silvan *et al*. (2007).** *Int J Syst Evol Microbiol* **57**, 2247-2258.

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